Design and Synthesis of Some Nitrate Derivatives of Mefenamic Acid with Expected Nitric Oxide Release

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Abstract

This study include design and synthesis of 2 derivatives of compounds consisting of mefenamic acid, glycine and organic nitrates (2-nitrooxy ethanol or 1,3-dinitrooxy-2-propanol). Nitric oxide NO has been reported to support many of the same mucosal protection mechanisms as prostaglandins and is sufficient for acute gastroprotection and ulcer healing. So we suppose these 2 compounds would reduce non-steroidal anti-inflammatory drugs NSAIDs gastrointestinal side effect.

Key words: Non-Steroidal anti-inflammatory drugs (NSAIDs), Glycine, Mefenamic acid, Organic nitrates, Nitric oxide (NO).

تصميم وتخليق بعض مشتقات النترات لحامض الميفينامك مع توقع تحرر لأوكسيد النتريك

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الخلاصة

تتضمن الدراسة تصميم و تخليق مركبين جديدين هما عبارة عن مشتقات حامض الميفينامك مع الكلايسين مع نترات عضوية (٢-نايتروكسي إيثانول أو ١,٣-دايناىتروكسي-٢-بروبانول). العديد من البحوث الحديثة تؤيد الدور المشابه بين أوكسيد النايتريك و البروستوكلاندين في حماية القناة المعوية و معالجة تقرحاتها. بناءا على ذلك نفترض بأن هذين المشتقين الجديدين سيساهمان في تقليل الأعراض الجانبية للأدوية غير الستيرويدية المضادة للإلتهاب على القناة المعوية.

الكلمات المفتاحية :الادوية المضادة للالتهابات الغيرستيرويدية (NSAIDs) ، كلايسين ، حامض الميفينامك ،نترات عضوية، اوكسيد النتريك (NO) .

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are still one of the first choices in the treatment of inflammatory conditions and among the most commonly prescribed drugs [(1)]. However, they are not devoid of adverse effects, gastrointestinal (GI) ulceration and renal damage being the most common, and even life-threatening [(2)]. Several strategies have been followed to reduce these adverse effects, including enteric coating, parenteral administration, and formulation of prodrugs that require hepatic metabolism for the cyclooxygenase (COX) activity to be unmasked and coadministration of either suppressors of acid secretion or exogenous prostaglandins (PGs), without the desired results [(3)]. A more recent and promising approach is that of COX-2 inhibitors [(4)]. But these drugs may lead to increased cardiovascular events [(5)]. Another approach to reduce the toxicity of NSAIDs is the linking of a nitric monoxide (NO) releasing moiety to these compounds, and thus, a new category of anti-inflammatory agents is emerging, the NO-NSAIDs [(6)]. Nitric oxide is a small diatomic radical that plays an important physiological role in nervous, cardiovascular, and immune systems [(7)]. Its generation is controlled by the three isoforms of NO synthase (NOS), neuronal and endothelial NOS (nNOS and eNOS), which are constitutive and produce nanomolar amounts of NO important for normal cell function and tissue protection, and inducible NOS (iNOS) which generates high amounts of NO responsible for the destruction of invading pathogens as part of an overall inflammatory response [(8)]. The endogenous tissue NO, generated constitutively by GI eNOS and nNOS, appears to play a key role in the chronic maintenance of gastrointestinal tissue integrity and in adaptive cytoprotection to injurious stimuli, perhaps acting synergistically with other cytoprotective prostaglandins [(9)]. NO promotes several gastric defense mechanisms by increasing mucus and bicarbonate secretion in the GI tract, increasing mucosal blood flow, and inhibiting the pro-inflammatory activities of neutrophils and platelets [(10)]. Additionally, NO may reduce inflammation connected to oxidative stress by scavenging reactive oxygen species, which can adversely increase mucosal permeability and kill cells [(8)]. Furthermore, it is found that NO and NO-derived reactive nitrogen species interact with peroxidases [(11)] and lipoxygenases [(12)], altering the generation of prostaglandins and leukotrienes, which are signaling molecules involved in inflammation [(13)].

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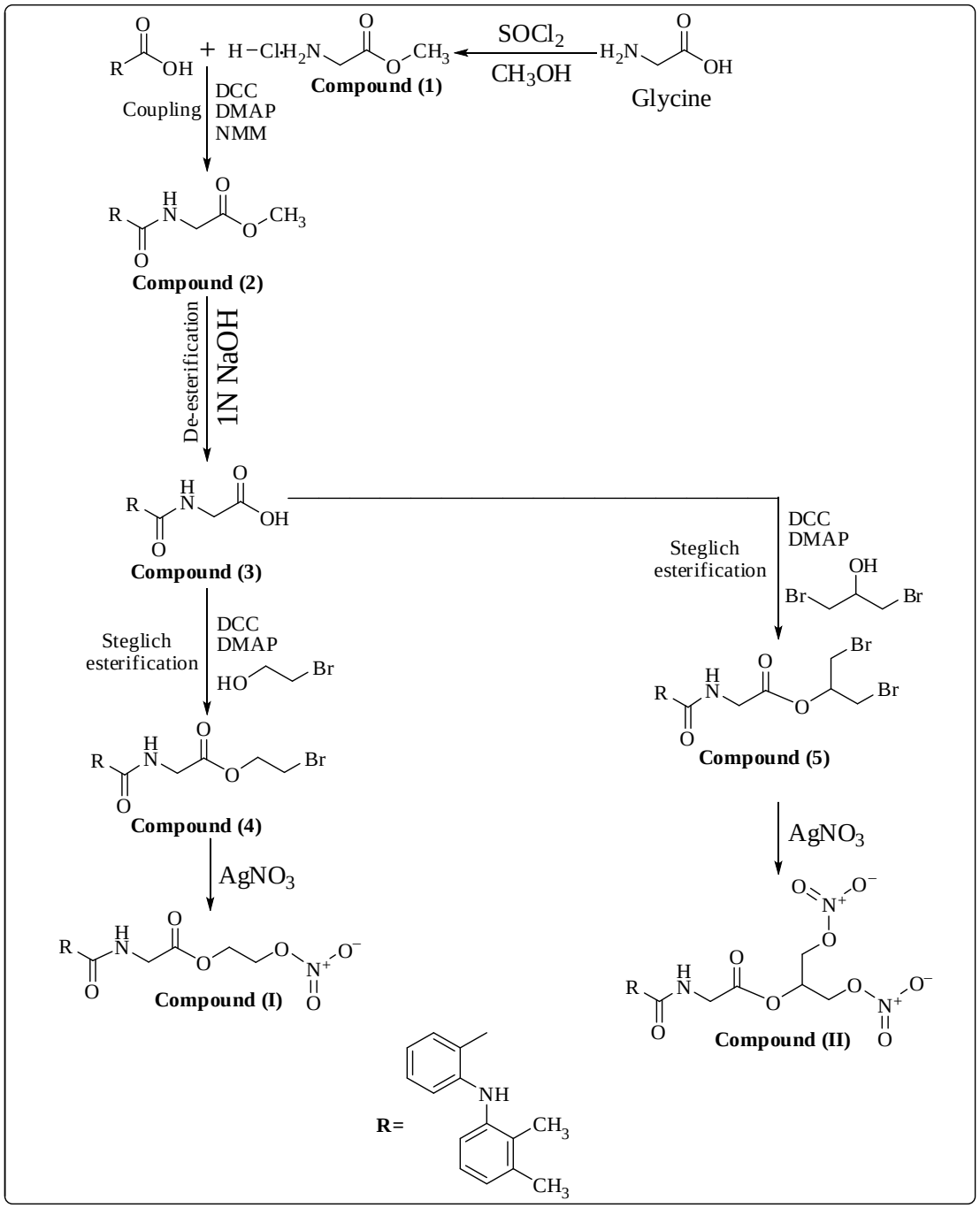
Several NO-NSAIDs, such as NO-aspirin, NO-diclofenac, NO-flurbiprofen, and NO-ketoprofen, have already been synthesized and clinical trials for some of them revealed protective activity against acute myocardial infarction [14]. These compounds appear to have a safe GI profile and can be effective in a variety of diseases including cardiovascular, rheumatological, lung diseases, Alzheimer's disease and cancer. There is also evidence to suggest that these compounds release NO in a metabolic, and not spontaneous, way and therefore, they do not interfere with blood pressure regulation [15]. Bearing in mind the above studies, we designed new derivatives of NO-releasing NSAIDs (scheme-1). Two derivatives of mefenamic acid-glycine esterified with 2-bromoethanol or 1,3-dibromo-2-propanol where they are then converted to nitrooxy derivatives upon treatment with silver nitrate in acetonitrile.



Scheme 1 : Chemical synthesis of compounds I and II.

Experimental

Chemicals

1,3-dibromo-2-propanol, 4-dimethyl amino pyridine (DMAP), N-methylmorpholine (NMM) (Aldrich), 2-bromoethanol, glycine, N,N′-dicyclohexylcarbodiimide (DCC) (Fluka), mefenamic acid (SDI), silver nitrate (Riedel-Dehaën), thionyl chloride (Merck), all solvents were of AnalaR type and used without further purification.

Detection

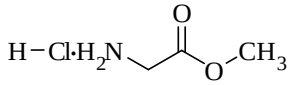
Melting points (uncorrected) were determined by capillary tube method on Barnstead/Electrothermal (UK) and FT-IR spectra were recorded on FT-IR spectrophotometer Shimadzu (Japan) at the college of science/ department of chemistry (Al-Mustansyria University). The CHNO analysis was carried out using Carlo-Erba 1106 Elemental analyzer (France). Ascending thin layer chromatography (TLC) was run on silica gel 60 F_{254} pre-coated aluminum sheets, Merck (Germany) to check the purity and the reaction's progress. The detection of derivatives was done using UV $_{254\ nm}$ lamp and the chromatograms were eluted by two solvent system:

A Ethanol: water (7:3) [16].

B Acetone: chloroform: acetic acid: water (3:2:1:4) [17].

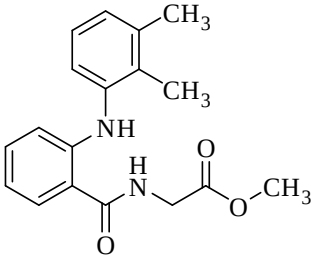
Synthesis

Synthesis of compound (1); (methyl-2-aminoacetate hydrochloride) [18]:



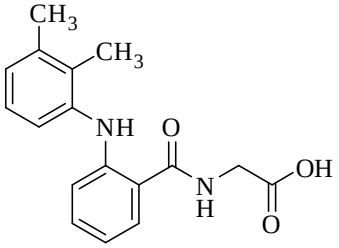
Thionyl chloride (3.25ml, 45 mmol) was slowly added by dripping to methanol (40 ml) kept on a temperature at (−10) to (−5°C); then glycine (3gm, 40 mmol) was added. The stirred mixture kept on 40-45°C for 2 hrs and then was refluxed for 4 h at 60-70°C under continuous stirring. Then excess thionyl chloride and solvent were removed under reduced pressure giving crude glycine methyl ester hydrochloride, which was treated with an 8 ml portion of cold diethyl ether at 0°C. The resulting solid product was collected and dried under vacuum. It was re-crystallized from hot methanol by slow addition of an 8 ml of diethyl ether followed by cooling at 0°C. The crystals were collected on the following day and washed twice with a (diethyl ether /methanol) mixture (5:1 ratio) followed by pure diethyl ether and dried under vacuum to give pure glycine methyl ester hydrochloride. The percent of yield is 95%. The physical appearance, melting point and the R_f value are listed in table (1) the characteristic IR bands are listed in table(1) the characteristic IR bands are listed in table(3).

Synthesis of compound (2); (methyl 2-(2-((2,3-dimethylphenyl) amino) benzamido) acetate) [19]:



To a stirred solution of compound 1 (1.883 gm, 15 mmol) in (120ml) of dimethyl formamide DMF, (3.3 ml, 30 mmol) of N-methylmorpholine (NMM) was added with stirring for 10 minutes, later on (3.62 gm, 15 mmol) of mefenamic acid was added, and the mixture was cooled down to (−10°C), then (0.183 gm, 1.5 mmol) of 4-dimethyl amino pyridine (DMAP) and (3.4 gm, 16.5 mmol) of N,N′-dicyclohexylcarbodiimide (DCC) were added with stirring which was continued for 2 days at 0°C and then at room temperature for 3 days. The reaction mixture was subjected to evaporation under vacuum to get rid of DMF, re-dissolved in 150 ml of chloroform from which the N,N′-Dicyclohexyl urea (DCU) was filtered off. The clear filtrate was washed with 0.5 N NaOH (3x150 ml), 1N HCl (3x75 ml), saturated sodium chloride solution (3x75 ml) and water (2x75 ml). The chloroform layer was dried over anhydrous sodium sulphate and the solvent was evaporated under vacuum. The percent of yield is 83.5%. The physical appearance, melting point and the R_f value are listed in table (1) the characteristic IR bands are listed in table(3).

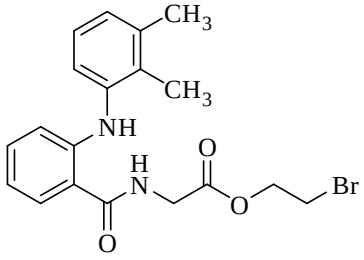
Synthesis of compound (3); (2-(2-((2,3-dimethylphenyl) amino) benzamido) acetic acid) [20]:



To a stirred solution of compound 2 (3.123 gm, 10 mmol) in 60 ml acetone, kept on 18-22°C, an aqueous solution of 1 N NaOH (25 ml) was added drop wise over a period of 30 minutes where the stirred reaction mixture was continued for additional 8 hours. The mixture was treated with 1 N HCl to get pH 1.

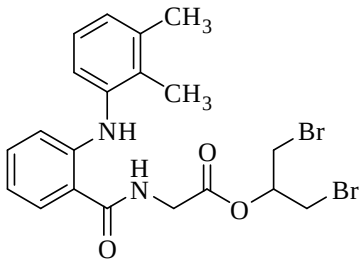
A precipitate was appeared which was separated by filtration, washed on the filter paper with water, and re-crystallized from n-hexane:acetone (4:3) mixture. The percent of yield is 53.5%. The physical appearance, melting point and the R_f value are listed in table (1), the characteristic IR bands are listed in table(3).

Synthesis of compound (4); (2-bromoethyl 2-(2-((2,3-dimethylphenyl) amino) benzamido) acetate) [21]:



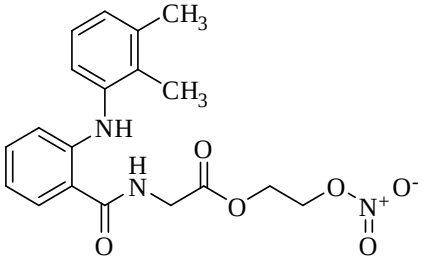
To a stirred solution of compound 3 (1.49 gm, 5 mmol), 2-bromoethanol (0.71 ml, 10 mmol) and DMAP (0.305 gm, 2.5 mmol) in (30ml) of dichloromethane (DCM) kept at −10°C, DCC (1.14 gm, 5.5 mmol) was added. The stirring reaction mixture was continued for 2 days at 0°C and then at room temperature for 3 days.The reaction mixture was subjected to evaporation under vacuum to get rid of DCM, re-dissolved in 50 ml ethyl acetate from which the N,N′-Dicyclohexyl urea (DCU) was filtered off. The clear filtrate washed with 5% sodium bicarbonate solution (3x25 ml), 1N HCl (3x25 ml) and water (2x25 ml). The ethyl acetate layer was dried over anhydrous sodium sulphate and evaporated under vacuum. The product was dissolved in ethanol (20 ml) with stirring. To the stirred solution about 20 ml of distilled water was added slowly from a dripping funnel. The precipitated solid was separated by filtration, dried and kept in a desiccator. The percent of yield is 82%. The physical appearance, melting point and the R_f value are listed in table (1) the characteristic IR bands are listed in table(3).

Synthesis of compound (5); (1,3-dibromopropan-2-yl 2-(2-((2,3-dimethylphenyl) amino) benzamido) acetate) [21]:



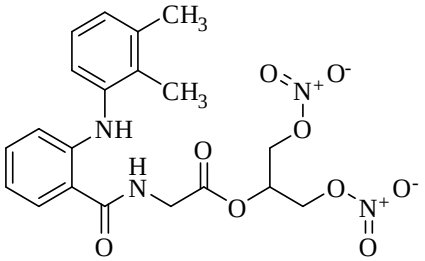
To a stirred solution of compound 3 (1.49 gm, 5 mmol), 1,3-dibromo-2-propanol (1.02 ml, 10 mmol) and DMAP (0.061 gm, 0.5 mmol) in (30ml) of DCM kept at −10°C, DCC (1.14 gm, 5.5 mmol) was added.The reaction mixture was worked up as in synthesis of intermediate 4. The percent of yield is 77%. The physical appearance, melting point and the R_f value are listed in table (1) the characteristic IR bands are listed in table(3).

Synthesis of compound (I); (2-(nitrooxy) ethyl 2-(2-((2,3-dimethylphenyl) amino) benzamido) acetate) [22]:



A solution of compound 4 (0.81 gm, 2 mmol) in dry acetonitrile (10 ml) was treated portion wise with a solution of $AgNO_3$ (1.36 gm, 8 mmol) in dry acetonitrile (20 ml) and the whole stirred mixture was refluxed for 48h in the dark. At the end of the reflux, the inorganic solid (black coloured silver bromide) was filtered off. The filtrate was subjected to evaporation under vacuum till dryness; the residue was dissolved in 20 ml DCM and washed with water (3x40 ml), saturated sodium chloride solution (2x40 ml) and finally water (2x40 ml). The organic layer was dried over anhydrous sodium sulfate, evaporated under vacuum and the obtained residue was re-crystallized from absolute methanol and triturated with petroleum ether (40-60°C). The percent of yield is 41%. The physical appearance, melting point and the R_f value are listed in table (1), the elemental microanalysis results are presented in table(2), and the characteristic IR bands are listed in table (3) .

Synthesis of compound (II); (1,3-bis (nitrooxy) propan-2-yl-2-(2((2,3-dimethylphenyl) amino) benzamido) acetate) [22]:



A solution of compound 5 (0.996 gm, 2 mmol) in dry acetonitrile (10 ml) was treated portion wise with a solution of $AgNO_3$ (2.72 gm, 16 mmol) in dry acetonitrile (40 ml) and the whole stirred mixture was worked up as in synthesis of compound I. The percent of

yield is 38%. The physical appearance, melting point and the R_f value are listed in table (1) , the elemental microanalysis results are presented in table(2), and the characteristic IR bands are listed in table (3) .

Result and Discussion

The synthesis of the designed compounds has been successfully achieved. And the purity and structural formulas of the synthesized compounds were characterized by their melting points, R_f values, FT-IR spectroscopy and elemental microanalysis.We expect these two compounds I and II would reduce gastrointestinal side effect largely. So we recommend for their biological evaluation for safe gastrointestinal profile.

Table 1 : The characterization and physical data of the synthesized compounds.

Compounds	Empirical formula	Description	Melting point °C	R_f value	Solvent system
Compound 1	$C_3H_8ClNO_2$	White powder	175-176	0.34	A
Compound 2	$C_{18}H_{20}N_2O_3$	Pale yellow powder	126-128	0.68	B
Compound 3	$C_{17}H_{18}N_2O_3$	Yellow powder	150-151	0.31	B
Compound 4	$C_{19}H_{21}BrN_2O_3$	Red crystal	156-158	0.75	B
Compound 5	$C_{20}H_{22}Br_2N_2O_3$	Red crystal	168-170	0.83	B
Compound I	$C_{19}H_{21}N_3O_6$	Brown powder	172-173	0.49	B
Compound II	$C_{20}H_{22}N_4O_9$	Brown powder	198-201	0.34	B

Table 2 : Elemental microanalysis of the compounds I and II.

Compound	Empirical formula	Molecular weight	Elemental analysis %		
			Element	Calculated	Observed
I	$C_{19}H_{21}N_3O_6$	387.387	C	58.91	58.13
			H	5.46	5.71
			N	10.85	10.33
			O	24.78	25.83
II	$C_{20}H_{22}N_4O_9$	462.41	C	51.95	50.37
			H	4.8	5.06
			N	12.12	12.59
			O	31.14	31.97

Table 3 : Characteristic FT-IR absorptions bands of the synthesized compounds.

Compound	Band (cm^{-1})	Interpretation
Compound 1	2500-3300	Asymmetrical and symmetrical NH_3^+ stretching
	1751	C=O stretching of ester
	1585, 1504	Asymmetrical and symmetrical bending of NH_3^+
	1261	Asymmetrical C-O stretching of ester
Compound 2	3377	N-H stretching of 2º aromatic amine
	3311	N-H stretching of 2º amide
	3063, 3037	Aromatic C-H stretching
	2931	Asymmetrical C-H stretching of CH_3 and CH_2
	2856	Symmetrical C-H stretching of CH_3 and CH_2
	1749	C=O stretching of ester
	1629	C=O stretching of 2º amide (amide I)
	1581	N-H bending of 2º amide (amide II)
	1510	N-H bending of 2º aromatic amine
	1276	C-N stretching of 2º aromatic amine
	1228	Asymmetrical C-O stretching of ester
Compound 3	3423	N-H stretching of 2º aromatic amine
	3367	N-H stretching of 2º amide
	2750-3200	OH stretching of carboxyl group
	3034	Aromatic C-H stretching
	2937	Asymmetrical C-H stretching of CH_2
	1741, 1707	C=O stretching of acid
	1626	C=O stretching of 2º amide (amide I)
	1579	N-H bending of 2º amide (amide II)
	1512	N-H bending of 2º aromatic amine
	1278	C-N stretching of 2º aromatic amine
	1211	Asymmetrical C-O stretching of acid

Compound 4	3308	N-H stretching of 2º amide
	3059	Aromatic C-H stretching
	2931	Asymmetrical C-H stretching of CH_3 and CH_2
	2856	Symmetrical C-H stretching of CH_3 and CH_2
	1747, 1705	C=O stretching of ester
	1639	C=O stretching of 2º amide (amide I)
	1583	N-H bending of 2º amide (amide II)
	1516	N-H bending of 2º aromatic amine
	1276	C-N stretching of 2º aromatic amine
	1224	Asymmetrical C-O stretching of ester
Compound 5	3275	N-H stretching of 2º amide
	3055	Aromatic C-H stretching
	2929	Asymmetrical C-H stretching of CH_3 and CH_2
	2856	Symmetrical C-H stretching of CH_3 and CH_2
	1745, 1710	C=O stretching of ester
	1631	C=O stretching of 2º amide (amide I)
	1581	N-H bending of 2º amide (amide II)
	1514	N-H bending of 2º aromatic amine
	1265	C-N stretching of 2º aromatic amine
	1224	Asymmetrical C-O stretching of ester
Compound I	3286	N-H stretching of 2º amide
	3061	Aromatic C-H stretching
	2931	Asymmetrical C-H stretching of CH_3 and CH_2
	2856	Symmetrical C-H stretching of CH_3 and CH_2
	1747, 1710	C=O stretching of ester
	1635	Overlapped C=O stretching of 2º amide (amide I) and N=O asymmetrical stretching of nitrate
	1581	N-H bending of 2º amide (amide II)
	1516	N-H bending of 2º aromatic amine
	1278	C-N stretching of 2º aromatic amine
	1226	Asymmetrical C-O stretching of ester
Compound II	3277	N-H stretching of 2º amide
	3059	Aromatic C-H stretching
	2929	Asymmetrical C-H stretching of CH_3 and CH_2
	2854	Symmetrical C-H stretching of CH_3 and CH_2
	1745, 1714	C=O stretching of ester
	1637	Overlapped C=O stretching of 2º amide (amide I) and N=O asymmetrical stretching of nitrate
	1579	N-H bending of 2º amide (amide II)
	1512	N-H bending of 2º aromatic amine
	1278	C-N stretching of 2º aromatic amine
	1226	Asymmetrical C-O stretching of ester

References

1. Smalley, W.E.; Ray, W.A.; Daugherty, J.R.; Griffin, M.R. Nonsteroidal Anti-inflammatory Drugs and the Incidence of Hospitalizations for Peptic Ulcer Disease in Elderly Persons. Am. J. Epidemiol., 1995, 141, 539-545.
2. Hawkey, C.J. Nonsteroidal anti-inflammatory drug gastropathy. Gastroenterology, 2000, 119, 521-535.
3. Wallace, J.L.; Cirino, J. The development of gastrointestinal-sparing nonsteroidal anti-inflammatory drugs. Trends Pharmacol. Sci., 1994, 15, 405-406.
4. Vane, J.R. Towards a better aspirin. Nature, 1994, 367, 215-216.
5. Mukherjee, D.; Nissen, S.E.; Topol, E.J. Risk of Cardiovascular Events Associated With Selective COX-2 Inhibitors. J. Am. Med. Assoc., 2001, 286, 954-959.
6. Wallace, J.L.; Reuter, B.; Cicala, C.; McKnight, W.; Grisham, M.B.; Cirino, G. A diclofenac derivative without ulcerogenic properties. Eur. J. Pharmacol., 1994, 257, 249-255.
7. Thomas, G. In medicinal Chemistry: An Introduction, 2nd ed.; Wiley-Interscience, UK, 2008, pp. 503-530.

8. Bandarage, U.K.; Janero, D.R. Nitric Oxide-Releasing Nonsteroidal Anti-inflammatory Drugs Novel Gastrointestinal-Sparing Drugs. Mini Rev. Med. Chem., 2001, 1, 57-70.
9. Ko, J.K.; Cho, C.H. Co-regulation of mucosal nitric oxide and prostaglandin in gastric adaptive cytoprotection. Inflamm. Res., 1999, 48, 471-478.
10. Wallace, J.L. Gastric ulceration: critical events at the neutrophile–endothelium interface. Can. J. Physiol. Pharmacol., 1993, 71, 98-102.
11. Clancy, R.; Varenika, B.; Huang, W.; Ballou, L.; Attur, M.; Amin, A.R.; Abramson, S.B. Nitric Oxide Synthase/COX Cross-Talk: Nitric Oxide Activates COX-1 But Inhibits COX-2-Derived Prostaglandin Production. J. Immunol., 2000, 165, 1582-1587.
12. Holzhütter, H.G.; Wiesner, R.; Rathmann, J.; Stösser, R.; Kühn, H. A Kinetic Model for the Interaction of Nitric Oxide with a Mammalian Lipoxygenase. Eur. J. Biochem., 1997, 245, 608-616.
13. Coffey, M.J.; Coles, B.; O'Donnell, V. Interactions of nitric oxide-derived reactive nitrogen species with peroxidases and lipoxygenases. Free Radic. Res., 2001, 35, 447-464.
14. Wallace, J.L.; Ignarro, L.J.; Fiorucci, S. Potential cardioprotective actions of no-releasing aspirin. Nat. Rev. Drug Discov., 2002, 1, 375-382.
15. Del Soldato, P.; Sorrentino, R.; Pinto, A. NO-aspirins: a class of new anti-inflammatory and antithrombotic agents. Trends Pharm. Sci., 1999, 20, 319-323.
16. Kushwaha, N.; Saini, R. K. and Kushwaha, S.K.S. Synthesis of some Amide derivatives and their Biological activity. Int.J. Chem.Tech. Res., 2011, 3, 203-209.
17. Rasheed, A.; Ashok Kumar, C. K. Tyrosine and glycine derivatives as potential prodrugs: design, synthesis, and pharmacological evaluation of amide derivatives of mefenamic acid. J. Enz. Inh. Med. Chem., 2010, 25, 804–811.
18. Brenner, M.; Huber, W. Herstellung von α-Aminosäureestern durch Alkoholyse der Methylester (Production of α-amino acid esters by alcoholysis of the methylester). Helv. Chim. Acta, 1953, 36, 1109–1115.
19. Zhao, M.; Bi, L.; Wang, W.; Wang, C.; Baudy-Floc'h, M.; Ju, J.; Peng, S. Synthesis and cytotoxic activities of β-carboline amino acid ester conjugates. Bioorg. Med. Chem., 2006, 14, 6998–7010.
20. Levit, G. L. ; Anikina, L.V.; Vikharev, Y. B.; Demin, A. M.; Safin, V. A.; Matveeva, T. V. and Krasnov, V. P. Synthesis and antinflammatory and analgesic activity of naproxen amides with amino acid derivatives. Pharm. Chem. J., 2002, 36, 232-236.
21. Neises, B.; Steglich, W. Simple Method for the Esterification of Carboxylic Acids. Angewandte Chemie International Edition in English, 1978, 17, 522 -524.
22. Abdel-Hafez, E.M.N.; Abuo-Rahma, G. A. A. G.; Abdel-Aziz, M.; Radwan, M. F. and Farag, H. H. Design, synthesis and biological investigation of certain pyrazole-3-carboxylic acid derivatives as novel carriers for nitric oxide. Bioorg. Med. Chem., 2009, 17, 3829–3837.